Evaluating Indicators of Seagrass Stress to Light

Running Head: Indicators of Seagrass Light Stress

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ABSTRACT

An optical water quality modeling approach is being developed in conjunction with physiological measures of low light stress in seagrasses to better predict their survival with water quality changes. The optical modeling approach focuses on decomposing the components of light attenuation through the water column into its constituents: total suspended solids (TSS), phytoplankton chlorophyll (chl a), and colored dissolved organic matter (CDOM). By determining the relative contributions of these constituents to light attenuation, the importance of each constituent can be evaluated. In a management-oriented approach, this allows for a direct link between constituent concentrations and attenuation. The photophysiological responses of seagrasses to chronic low light stress are required in the optical model to determine the threshold levels below which plant growth or survival are no longer supported. Photosynthetic fluorescence yield is a rapid, non-destructive technique to evaluate plant stress, and has recently been demonstrated to track recovery of seagrasses to acute stress events. Experimental results suggest that this approach is less ideal for slow, chronic changes that occur as water clarity declines, because seagrasses have the capacity to acclimate to the reduced ambient light conditions.

INTRODUCTION

Estuaries and coastal waters are highly productive, ecologically- and societal-valuable ecosystems. They are under increasing stress from both anthropogenic factors, such as nutrient enrichment and sedimentation, in addition to altered frequencies and intensities of natural disturbances arising from global climate change. Seagrasses are dominant primary producers that play a central role in the stability, nursery function, biogeochemical cycling and
trophodynamics of coastal ecosystems, and as such, are important for sustaining a broad spectrum of organisms (Thayer et al., 1984; Hemminga and Duarte, 2000). For example, they stabilize sediments, which are easily resuspended if the plants are lost, resulting in increased and prolonged turbidity that reduces available light reaching the seafloor. For these reasons, seagrasses are widely recognized as ‘barometers’ of estuarine water quality, being perhaps the most parsimonious integrator of estuarine water quality throughout the range of their current and historic distribution (Dennison et al., 1993). Thriving seagrass communities signal a productive, diverse and biogeochemically-trophically well-coupled coastal ecosystem (Harlin and Thorne-Miller, 1981; Thayer et al, 1984; Fonseca et al., 1998; Hauxwell et al., 2001). Accordingly, the presence or absence of seagrass is a useful measure of estuarine condition, but reliance on presence/absence as an indicator implicitly requires significant degradation of estuarine water quality (Zimmerman et al., 1991; Short and Wyllie-Echeverria, 1996). By focusing on seagrass decline, we are restricted to detecting conditions when water quality is already so degraded that there is virtually no time for corrective actions. Therefore, early detection of sub-lethal stress thresholds in seagrass plants is crucial for effective conservation of this resource.

The role of seagrasses as indicators of estuarine condition, particularly decreased water clarity, was proposed in the early 1990’s (Kenworthy and Haunert, 1991; Neckles, 1994). Dennison et al. (1993) concluded that seagrasses were potentially sensitive indicators of declining water quality because of their high light requirements (15-25% surface irradiance) compared to those of other aquatic primary producers, such as macroalgae and benthic microalgae, with much lower light requirements (Markager and Sand-Jensen, 1992, 1996; Agusti et al., 1994). To develop predictive indicators of estuarine suitability for seagrasses, both water-quality driven stressors and the whole-plant integrated responses should be assessed. Potential
predictive indicators need to respond clearly and reliably to abiotic factors that cause sub-optimal seagrass growth (e.g., light limitation) and should come from a suite of approaches over a range of hierarchical levels.

1. Bio-optical Modeling

Provided that all other environmental parameters are suitable, the light environment during the growing season is the most important abiotic factor determining survival of seagrasses (Moore et al., 1997; Batiuk et al., 2000; Dixon, 2000a). Light attenuation by the water column is a major variable related to seagrass decline (Dennison and Alberte, 1982, 1985; Bulthuis, 1983). Another is epiphytic light attenuation, which Dixon (2000b) found to be more important in the subtropical waters of Florida. Low light levels, below some minimum physiological requirement (typically 15-25% of incident surface light = Io) may result in a loss of seagrasses. Light is attenuated down the water column resulting in less light available at the bottom (Iz) than at the surface (Io) by factors including:

1. turbidity, expressed as total suspended particulate matter (SPM),
2. phytoplankton, which both absorb and scatter light, expressed in chlorophyll concentration (chl a),
3. colored dissolved organic matter (CDOM) leaching from decaying vegetation,
4. macroalgae and epiphytic microalgae that grow on the seagrass. These are usually more problematic when eutrophication is taking place (Harlin and Thorne-Miller, 1981; Hauxwell et al., 2001).

Light attenuation (Fig. 1A) can be expressed as PLW (Percent Light through Water) or the combined effects of contributions due to turbidity, chlorophyll, and color, resulting in
significantly reduced light with increasing depth. Additional light attenuation can occur at the leaf surface due to epiphyte fouling, PLL (Percent Light at Leaf), which occurs primarily under heavily eutrophic conditions (Batiuk et al., 2000). However, this may not always be the case. For instance, Dixon (2000b) found light attenuation due to epiphytes averaging 34% of all attenuation of light in lower Tampa Bay where chl \( a \) has an annual average that is less than 5 mg m\(^{-3}\) (not heavily eutrophic). One of the goals of our research has been to refine a bio-optical water quality model (Gallegos, 1994; 2001) by determining the importance of SPM, chl \( a \), and CDOM on light attenuation to seagrasses in North Carolina during different seasons.

Graphically, the results of the bio-optical model are shown as in Fig 1B, where two components of light attenuation (chl \( a \), TSS) are presented on the X-Y axes. The third constituent, CDOM, can be plotted on the Z-axis on a 3D plot to show all three components of attenuation. Median concentrations for one water quality sample are plotted on this graph and compared to a minimum light water quality requirement for a given depth (for simplicity, depicted as a line of constant attenuation), which is calculated using a radiative-transfer model and knowledge of seagrass species light requirements. Target minimum water-clarity requirements for seagrass survival are found at the intersection of vectors perpendicular to the axes or the origin from the median sample concentration. The target concentrations in this figure suggest that both TSS and chl \( a \) need to be reduced to meet the minimum light requirements of this seagrass species.

Determining the minimum light requirements for a given species requires either monitoring and identifying the deep edge of existing seagrass beds within an estuary, or an experimental approach where the minimum light requirements for survival are determined over a period of
time. An understanding of the physiological processes occurring within plants subject to chronic light-limitation is, therefore, important when applying the bio-optical model.

2. Plant Physiology

For a seagrass-dominated ecosystem to be judged sustainable, the seagrasses must be vital, and capable of long-term persistence by growth and reproduction. We are focusing on a common set of photosynthetic and physiological metrics that measure seagrass condition and may provide early warning of plant stress or demise, before extensive loss of seagrass occurs.

A promising approach needing further investigation is chlorophyll fluorescence of photosystem II, also known as P680, where a molecule of water is split to form O$_2$ and a reductant in the light reaction of photosynthesis (Stryer, 1981). To measure this, we utilized a photosynthetic efficiency analyzer (PEA) that was developed to measure the health of crops (e.g., Critchley and Smilie, 1981; Havaux and Lannoye, 1983; Bowyer et al., 1991; Filiault and Stier, 1999) and because all plants utilize the same fundamental processes in photosynthesis, this instrument can also be used on marine macrophytes.

The PEA consists of a computer and a photo emitter/sensor unit. Clips are supplied with the apparatus, these are attached to a plant leaf and serve to occlude all but a small area of the leaf needed for photosynthetic testing. A small area of the plant leaf is dark-adapted with the clip and then a shutter built into the clip is opened, exposing the leaf area under the clip to high intensity light provided by nearly monochromatic light emitting diodes (LED) located in the photo emitter/sensor unit. The chlorophyll in the dark-adapted area of the leaf fluoresces and the PEA measures this. The initial fluorescence ($F_o$) and the maximal fluorescence ($F_m$) are recorded and the difference between the maximal and initial fluorescence levels ($F_m-F_o$) is called the variable
fluorescence ($F_v$). The PEA computer calculates the ratio $F_v/F_m$, or photosynthetic efficiency; the greater the fluorescence per unit incoming light, the higher the efficiency of the photosystem which equates with a plant under low physiological stress – conversely, low $F_v/F_m$ indicates a plant under stress (Schulze and Caldwell, 1990; Krause and Weis, 1991; Rohacek and Bartak, 1999).

This approach has been used to measure acute stress in seagrasses, such as desiccation (Adams and Bates, 1994; Bjork et al., 1999), temperature or salinity shifts (Ralph et al., 1998; Ralph, 1999), and even changes in ambient light over short time durations (Beer and Bjork, 2000; Major and Dunton, 2002). However, to our knowledge this technique has not been evaluated in seagrass plants subject to chronic stress, such as light limitation arising from reduced water clarity.

There are two components to our research that will be discussed here:

1. Monitor water quality to calibrate a bio-optical model and using this modeling approach to understand water quality changes that result in low-light stress
2. Determine seagrass photophysiological indicators as a measure of individual plant responses to light stress.

METHODS
1. Calibrating a Bio-optical Model

To refine the bio-optical model as an indicator for seagrass habitat suitability in the Mid-Atlantic region of the eastern coastal United States, we have initiated a monthly water-sampling program in North River, North Carolina, an estuary that contains sizeable seagrass beds along a gradient in water clarity. We are collecting water samples at nine stations in North River, North
Carolina (Fig 2). Additionally, at each station we are profiling water quality with YSI 6600 multi-parameter probes (temperature, salinity, DO, pH, turbidity, and chlorophyll fluorescence), as well as collecting light attenuation data using a LICOR 4π sensor. The light data are used to calculate attenuation coefficients to compare with Secchi disk readings and laboratory measured optical properties of the water samples. Water samples are analyzed for total absorption (referenced to pure water) and scattering coefficients using the methods described in Gallegos (1994, 2001).

2. Light-Stress Experiments

A light gradient (Table 1) was created in a large indoor tank under controlled environmental (light, air temperature) conditions to determine minimum integrated light requirements for seagrass survival. A bank of overhead Halogen® lights of increasing wattage provided the different light treatments. Lights were placed on a timer to create a 12:12 L:D environment. Water was provided as a flow-through system and temperature was monitored at 30 minute intervals at both ends of the light gradient. Water quality data were collected with a YSI® 6000 multi-parameter water-quality probe. Two seagrass species were tested in 2003: Zostera marina (eelgrass) seedlings were grown for 20 weeks (27 Jan – 6 Jun 2003) and Halodule wrightii (shoalgrass) plants were grown for 16 weeks (8 Jul – 27 Oct 2003) in a range of light intensities (Table 1).

Two tubs (Rubbermaid® #2951) were planted with nine Z. marina seedlings or nine H. wrightii plants (3x3 arrangement) and placed into each of the seven light gradient treatments after allowing ten days recovery from transplant stress. Initial morphological measurements were taken on the day the plants were placed into the light gradient tank and weekly thereafter.
until the experiment was terminated. Biweekly, mature leaves from a random subset of plants were collected to measure width and destructive analysis of tissue constituents, including chlorophyll extraction and tissue nutrient content (C:N ratio) using standard analytical methods.

For the weekly chlorophyll fluorescence measurements, the second leaf on the terminal apical shoot was clipped about half way along the adaxial side as per the recommendations of Durako and Kunzelmann (2002). Dark adaptation of the clipped portion was ten minutes and the PEA recorded chlorophyll fluorescence from the leaf for one second. Typically the maximum fluorescence in green plants occurs within the first second after exposure to the high intensity LED lights (Srivastava et al., 1999).

RESULTS

1. Water Quality Monitoring; North River, North Carolina

Temperature followed an approximate sinusoidal pattern during the year with minimum temperatures in February and maximum temperatures in July and August (Fig. 3). Shallower upriver stations had more rapid temperature changes than the tidally influenced downstream stations, which reflected oceanic water temperatures. Salinity was higher and more stable in the downstream, oceanic influenced section of North River than the upstream portion, which was more influenced by terrestrial runoff from surrounding salt-marsh drainage streams. Salinity dropped after heavy rainfall events, this was especially evident during the wet spring and summer of 2003 (Fig. 3); this year was the wettest on record with 2337mm (92 inches) recorded, 50% greater than average (National Weather Service data).

Chlorophyll concentrations (mg m\(^{-3}\)) were lowest during the cold winter months and increased during the spring and summer when phytoplankton were more abundant (Fig. 3).
Highest values were observed at the farthest upstream station corresponding with increased N-concentrations measured at this station. High chlorophyll concentrations were also recorded during storm events and co-occurred with elevated turbidity, likely due to resuspension of benthic microalgae (Fig. 3). Turbidity was similar at all stations during a given sampling date. In general, there was a trend of increasing turbidity from the downstream to the upstream stations (Fig. 3). Turbidity was lower in winter than other seasons. The highest turbidity values were recorded at shallow upstream sites during storms (e.g., Sept 2002).

The effect of chlorophyll and turbidity on measured light penetration to the bottom at each of the nine stations was expressed as an attenuation-coefficient ($K_d$), derived from the negative exponential Beer-Lambert function. Higher values of $K_d$ indicate more light attenuation (i.e., more turbid water). $K_d$ values increased with distance from the Beaufort Inlet at station 1 to the highest values found at station 9, where chl $a$ and turbidity were also typically highest. During two months (Sept 2002 and Mar 2003) samples were collected during storm conditions (tropical storm and northeaster respectively) with some of the highest attenuation values seen, indicating the importance of natural events in driving extreme values in this estuary. For seagrass survival to 1 m, $K_d$ should not exceed 1.5m$^{-1}$, i.e., 22% of surface light (Io) reaching the bottom, as indicated by the dotted line (Fig. 4). This condition was typically met at the stations near where seagrass are found in North River (stations 2 through 6). $K_d$ values sufficient for seagrass survival were less frequently seen in the upstream stations.

Using our initial calibration values from the bio-optical model, monthly water quality conditions were plotted for North River sampling stations (Fig 5). Most samples fall in the region of acceptable light quantities ($\geq$22% Io) reaching 1m depth, except for a few samples that were collected during storm conditions (Sept 2002 and Mar 2003). This indicates that water
clarity in the North River is sufficient to support seagrasses to 1m depth and this agrees with observations made on the multi-decadal persistence of seagrass beds in this estuary (since early 1970’s). Interestingly, the line of constant attenuation for the North River (solid line) allows much higher turbidity concentrations than the same light-at-depth requirement for Chesapeake Bay seagrasses (dashed line). The reason for this (based on initial results) is that per unit mass suspended sediments in the North River absorb and scatter much less light than those in the mesohaline Chesapeake Bay, due to differences in sediment properties related primarily to particle size (and perhaps also phytoplankton composition) between these regions.

Additionally, the North Carolina state estuarine water-quality criteria of 40mg m$^{-3}$ chl $a$ and 25NTU turbidity (NC-DENR, 2003) are shown on the plot (Fig. 4, black box). Almost all water quality samples from North River fall within the acceptable State criteria, and within the SAV-survival criteria based on the bio-optical model calibrated to North River conditions. There are combinations of turbidity and chlorophyll concentration permitted by State criteria that would not support seagrasses according to the bio-optical model (Fig. 5, shaded triangle); but interestingly, no water quality observations fell in this region.

The results from the bio-optical model can be corroborated by results from the permanent monitoring stations. Three locations in North River are designated as permanent stations, with continuous unattended water quality and light monitoring using YSI® and LICOR® sensors and data loggers. These locations are near stations 2, 6, and 9 in North River (Fig. 2). Station 6 has been continuously monitoring since September 2002. Integrated daily light (mol m$^{-2}$ d$^{-1}$) received at two sensor depths, 1 and 1.5 meters, was averaged for each month and the number of hours that irradiance exceeded the photosynthesis saturation intensity (150 µmol m$^{-2}$ s$^{-1}$) for Z. marina (Dennison and Alberte 1982) calculated (Table 2). Light available for photosynthesis
was high in Dec-Feb (clear water) and Jun-Jul (strong solar irradiance) even though summer time water clarity was much reduced compared to the winter condition, i.e., stronger solar irradiance may compensate for higher chl a concentrations in the summer.

2. Light Stress Experiment

Comparing and contrasting the results of the light gradient experiments using Zostera marina seedlings and Halodule wrightii plants, showed similar responses by both species to light limitation stress. Both instantaneous and integrated (12hr) irradiance fluxes during the two experiments are given in Table 1, as well as integrated irradiance greater than the saturation irradiance (approx. 150µmol) and compensation irradiance (approx. 10µmol) for Z. marina (Dennison and Alberte, 1982) and H. wrightii.

For both species, all but the two greatest light treatments resulted in a deficit of light to saturate photosynthesis, ultimately resulting in mortality of many of the plants. This result indicates that irradiances consistently lower than the saturation threshold will ultimately result in plant death. Observations made during weekly measurements suggested that mortality in response to light-limitation occurred from the base of the plant upward, with the meristem becoming non-viable about 1-2 weeks before the leaves were apparently dead (Z. marina) or broke off at the sheath (H. wrightii). This observed lag-time of 1-2 weeks may be critical in monitoring seagrass mortality under natural situations where light-limitation is the cause of mortality.

During the Z. marina experiment, water temperatures increased from 10 to 28°C. The upper favorable temperature for this species is around 25°C (Thayer et al., 1984), which was attained in May (Fig. 6A). This high temperature stress resulted in reduced growth, as seen by a reduction in
leaf area for the remainder of the experiment. In contrast, temperatures started to decline during the *H. wrightii* experiment (Fig. 6B). They remained at 28°C until early September, then temperatures declined to 18°C by the end of the experiment.

Plant growth and photosynthesis responses for *Z. marina* and *H. wrightii* in the light gradient experiment showed some similarities as well as differences with respect to the minimum integrated light requirements for survival. Both single shoot *Z. marina* seedlings and two shoot *H. wrightii* plants were grown for the duration of their growth season. For *Z. marina*, no branching or spatial expansion was observed in any of the seven irradiance treatments. Five of the 117 seedlings flowered (4.3%) all at irradiances of 33 µmol or higher (Fig 7A). In *H. wrightii* plants, branching or spatial expansion was observed in the highest irradiance treatment only (Fig. 7B). Shoot loss and/or mortality were observed at irradiances of 60 µmol or lower in this species. These integrated light thresholds indicate that tropical *H. wrightii* plants have somewhat higher minimum light requirements for survival than temperate *Z. marina* seedlings.

The length of the longest leaf on each surviving seedling was measured as a proxy for canopy height in both species. In *Z. marina*, leaf growth (linear extension) occurred only in the two highest light levels (irradiance > saturation). Early mortality (leaf length = 0) was observed in the two lowest light levels with evidence of plant stress by weeks 3 and 5 respectively, about half the time to mortality (Fig. 7C). In *H. wrightii*, leaf length was maintained only at the two highest light levels (irradiance ≥ 95 µmol). Early mortality was observed in the lowest light level with evidence of plant stress by weeks 3-4, about half the time to mortality of all the plants in this treatment (Fig. 7D).

Leaf area (single surface) was calculated for the strap-like leaves by multiplying maximum length by average width in both species. Width was measured on alternative sampling dates on a
random subset of 3-5 plants with linear interpolation for non-sampled dates. Area was primarily influenced by leaf length, as width remained approximately constant within a given light treatment (Figs 7E&F).

*Z. marina* seedling photosynthesis was measured with an OptiSciences® OS-30 PEA (Plant Efficiency Analyzer) and *H. wrightii* photosynthesis was measured with a Hansatech PEA that had increased sensitivity, an important consideration given the narrow leaves of this species. Photosynthetic yield, expressed as the ratio $F_v/F_m$, ranged from 0.6-0.75 in healthy *Z. marina* seedlings (Fig. 7G) and from 0.65-0.78 in healthy *H. wrightii* plants (Fig. 7H). In both species $F_v/F_m$ fell below 0.5 in plants that were dead or dying. No significant difference in $F_v/F_m$ was observed in those plants remaining alive across all seven light treatments, indicating acclimation of the photosynthetic apparatus to the ambient light field and temperature conditions. In summary, the yield ratio ($F_v/F_m$) is not a sensitive measure of chronic stress response to light or temperature for which tropical *H. wrightii* and temperate *Z. marina* can acclimate.

**DISCUSSION**

Seagrass is an important estuarine habitat that is declining globally (Green and Short, 2003). Much of this decline can be attributed to decreased light availability to the plants because of reductions in water clarity due to degrading water quality (Dennison et al., 1993; Hauxwell et al., 2001). Seagrasses have relatively high light requirements compared to other marine primary producers and so are more susceptible to low light stress (Kenworthy and Haunert, 1991; Kenworthy and Fonseca, 1996; Dixon, 2000a). For this reason, they have been proposed as indicators of estuarine change (Dennison et al., 1993). However, it is desirable to have an
indicator(s) that provides an early warning of potential seagrass demise, rather than waiting until after the fact.

Water quality criteria, particularly those related to the optical water quality (i.e., water clarity) needed for the survival and growth of seagrasses have been the subject of considerable research (Kenworthy and Haunert, 1991; Neckles, 1994; Gallegos and Kenworthy, 1996; Kenworthy and Fonseca, 1996; Longstaff et al., 1999; Zimmerman, 2003). A general conclusion of those workshops and research programs was that water-column clarity needs to be greatly increased in order to provide light conditions suitable for the survival of most seagrasses (Kenworthy and Fonseca, 1996; Moore et al., 1996; Batiuk et al., 2000).

1. Water Quality Stress Indicators

We are developing a water-quality stress-indicator using a bio-optical model of water clarity that can identify harmful trends in water quality and make explicit what is causing the increased light attenuation. This will enable early preventative management actions to be taken to remediate water quality before it becomes critically limiting to seagrass survival, resulting in loss of this important habitat.

The bio-optical model is useful because it permits us to determine the relative contributions of the different water quality parameters to light attenuation at different positions in the estuary. This is a very important step in managing water quality as it makes explicit the relative contribution of suspended particles, phytoplankton, and color to light attenuation. From this knowledge, target reductions or target loading rates for one or more of these components of water quality can be set to achieve a desired water clarity. Comparing light attenuation calculated at the deep survival limit of seagrasses with water quality concentrations measured
there allows the determination of ranges of water clarity that permit expansion or cause contraction of the seagrass bed. Basing the model on inherent optical properties has the advantage that extrapolation beyond the range of water quality concentrations encountered during model development is possible, because the absorption and scattering coefficients are linearly related to the relevant water quality concentrations (Fig. 1B). Such an exercise can be used to determine the availability of light at the edge of the seagrass bed in response to hypothetical scenarios, such as accelerated eutrophication resulting from increased nutrient loading in the watershed. This bio-optical model has already been calibrated to conditions typical for Chesapeake Bay, Maryland (Gallegos, 2001) and Indian River Lagoon, Florida (Gallegos and Kenworthy, 1996), and recently North River, North Carolina (Biber and Gallegos, unpubl. data); all estuaries with significant seagrass habitats.

Based on the initial results of the bio-optical model for North River seagrass habitats, we can conclude that even though water quality appears to be adequate to sustain stable seagrass beds in this estuary, the long-term survival of these mostly shallow seagrass communities in North River is still uncertain, especially when considering the likelihood of sea-level rise projected to occur within the next century. More stringent water clarity criteria with respect to seagrass habitats should to be adopted by the State to ensure seagrasses receive adequate light. Similar results have been found by researchers in Chesapeake Bay, where large declines in SAV have been ongoing and are almost impossible to reverse (Orth and Moore, 1983; Dennison et al. 1993; Batiuk et al., 2000; USEPA, 2003).

Seagrass communities in North Carolina present a unique situation at the overlap of temperate and tropical biogeographic regions resulting in the coexistence of *Zostera marina* (temperate, winter dominant) and *Halodule wrightii* (tropical, summer dominant), allowing
research on the competitive interactions between these two widely distributed seagrasses (Green and Short, 2003). Results are likely to be conservative as plants may already be experiencing some stress related to their presence at extremes of distribution. Further differences exist in that *Z. marina* reproduces sexually with annual seedling recruitment being a significant contribution to the population, whereas *H. wrightii* has never been observed to reproduce sexually in North Carolina, dispersing instead by clonal growth and asexual fragmentation (Thayer et al., 1984; Ferguson et al., 1993). These different reproductive strategies affect the dispersal ability and population recovery strategies after severe stress events, with potentially important repercussions in the face of possible global climate change scenarios. Because of the extreme importance of annual seedling recruitment to eelgrass population dynamics, we suggest that future research be focused on understanding the stress-tolerance of this life-stage, so that relevant differences in survival ability can be incorporated into management plans.

We suggest that the higher irradiance penetrating through the water column in the winter months, when water is clearer, may be a critical window of opportunity for seedling growth for *Z. marina*. Similarly for *H. wrightii*, high light levels, due to strong solar irradiances, in early summer may be critical for this species to grow rapidly early in the season, even though summer water clarity is much reduced compared to the winter condition. Similar critical windows of increased irradiance, due to higher water clarity have been identified in the polyhaline Chesapeake Bay, from Mar1 – May31, the early part of growing season for *Z. marina* (USEPA, 2003). Management implications of this are that better water quality needs to be present during these critical periods.

2. Plant Physiology Indicators
Most current approaches do not address the integrated light requirements of seagrass, focusing instead on “instantaneous” measures of irradiance flux and seagrass photosynthetic rates, e.g., photosynthesis-irradiance curves (Dennison and Alberte, 1982, 1985; Goodman et al., 1995; Zimmerman et al., 1995; Bintz and Nixon, 2001). This approach implicitly ignores cumulative stress effects, so that important questions regarding how the duration of exposure or the frequency of exposure to a given level of environmental degradation might influence survival of the seagrasses are overlooked. Only recently has the frequency and duration of stressful conditions started to be investigated for the survival of seagrass (Dunton and Tomasko, 1994; Onuf, 1996; Moore et al., 1997). It is this whole plant integrated response that is biologically and ecologically relevant to seagrass management. This is also the scale at which we can link water quality to seagrass photophysiology.

Recent technological advances and research into plant photosynthesis have resulted in the commercial production of instruments that can measure chlorophyll fluorescence to detect sub-lethal stress thresholds in situ, even underwater (e.g., Diving PAM, Walz, Germany). This technique has opened a new vista for seagrass physiologists, allowing rapid, non-destructive measurements of plant photosynthetic performance (e.g., Ralph and Burchett, 1995; Beer et al., 1998; Ralph et al., 1998; Bjork et al., 1999; Beer and Bjork, 2000; Durako and Kunzelman, 2002).

In our light-limitation experiments, seagrass chlorophyll induction kinetics were measured using a PEA. Contrary to expectation, the photosynthetic efficiency (Fv/Fm) indicated that both \( Z. \ marina \) and \( H. \ wrightii \) adapt their photosystem to the ambient light and temperature conditions within less than a week, as indicated by a near constant Fv/Fm ratio. Because of this adaptation, declines in the Fv/Fm ratio in low light treatments failed to precede losses in shoot
number (c.f. Fig. 7A, G). Fv/Fm ratio alone is, therefore, unlikely to be a suitable tool to measure sub-lethal chronic stress responses in seagrass because of its invariance in the face of photosystem change. However, other ratios and indices derived from chlorophyll fluorescence induction kinetics that can be collected by PEA show more promise.

Chlorophyll fluorescence induction kinetics are extremely rich in terms of quality and quantity of different information (Strasser et al., 1995) and can only be measured by a plant efficiency analyzer (PEA) with a 1µs sampling rate or higher, but not a pulse amplitude modulated (PAM) fluorometer, which has only a 30 µs sampling rate, as the lower sampling frequency misses the very early induction dynamics (Rohacek and Bartak, 1999). Because the induction kinetics are determined by both the physiological state of a plant and the ambient and past physical and chemical environmental conditions, they can be used to detect and predict sub-lethal stress thresholds not apparent in Fv/Fm. Strasser et al. (1995, 1996) have developed a method (JIP-test analysis) to further analyze the data on chlorophyll fluorescence induction kinetics gathered by PEA into ratios and indices of photosynthetic performance of the photosystem.

Chlorophyll exhibits upon illumination a fast fluorescence rise from an initial fluorescence intensity, \( F_0 \), to a maximal intensity, \( F_P (=Fm) \). Between these two extremes, the fluorescence intensity usually shows two intermediate steps: \( F_J \) at about 2 ms, and \( F_I \) at about 30 ms (Strasser and Govindjee, 1992a, 1992b; Strasser et al., 1995) followed by \( F_P \) at about 300 ms. The labeling of these steps follows an alphabetic order, from the initial (\( F_0 \)) to the final (\( F_P \)) part of the transient. Based on the information inherent in the O-J-I-P fluorescence transient, a test has been developed (called "JIP-test" after the steps of the transient), which can be used as a tool for rapid
screening of many samples providing additional information about the structure, conformation, and function of their photosynthetic apparatus (Strasser and Strasser, 1995; Strasser et al., 1996).

As seagrass leaves age, many of the JIP-test ratios and indices change (Fig. 8). Standardized methods for collecting PEA data for JIP-test analysis from seagrasses have not yet been adopted, in part because this is a new approach, and in part due to the lack of knowledge on the variability inherent in natural, healthy seagrass populations (Fig. 9). To better understand seagrass photophysiology, PEA and JIP-test will need to be further investigated to determine the inherent variability of these ratios and indices in seagrass species under stress (e.g., light-limitation). We will continue to test the responses of *Z. marina* and *H. wrightii* to chronic light-stress using PEA and JIP-test analyses to determine whether this may provide potentially powerful early warning of imminent demise of seagrass plants.

The ability to combine both water quality stressors, i.e., the light environment linked explicitly to constituents of attenuation, with plant physiological responses to light stress forms the basis of an integrative indicator of water quality that will permit evaluation and determination of the suitability of water quality in an estuary for continued seagrass sustainability.

CONCLUSIONS

Once the optical properties of the bio-optical model are calibrated to regional conditions, the next step is to utilize the seagrass ecophysiological information to set light requirement thresholds for seagrass plant survival, growth, and reproduction. Each of these three thresholds will be successively greater than the previous one because of the increased cumulative light resource requirement by these plants to achieve the successive stages of development, i.e., resources (light) required for growth, a net increase in biomass, will be greater than those
required for survival (maintenance metabolism or no net increase in mass) and similarly for growth to reproductive maturity. The final stage of this process will be to use the bio-optical model as a tool to forecast the results of possible altered water quality scenarios and determine the impacts of these changes on existing seagrass habitats.

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Grateful thanks to the cadre of persons, who were instrumental in assisting with data collection especially in the field, including: Christine Addison, Craig Bonn, John Burke, John Brewer, Priscilla Delano, Teri Denault, Don Field, Nikki Fogarty, Tom Gallo, Kamille Hammerstrom, Kar Howe, Loretta Leist, Amit Malhotra, Ryan McQuinn, Joe Purifoy, Karen Rossignol, Stopher Slade, Amy Uhrin, Paula Whitfield, Pam Wyrick, and Rich Weaver. Additional support was provided by the Center for Coastal Fisheries and Habitat Research, NCCOS, NOS, NOAA, Beaufort, N.C. Thanks to Glen Thursby for providing the tools to do the JIP-test analyses. This research was funded by EPA-STAR grants (R82867701) to the ACE-INC EaGLes Center (www.aceinc.org) and (R82868401) to the Atlantic Slope Consortium EaGLes Center (www.asc.psu.edu).

REFERENCES


Table 1: Seven irradiance treatments used for indoor light experiment, expressed as instantaneous irradiance, integrated daily irradiance for a 12 hour day, number of hours (Hsat) that irradiance exceeded a saturation intensity (150µmol m$^{-2}$ s$^{-1}$) and number of hours (Hcomp) that irradiance exceeded a compensation intensity (10µmol m$^{-2}$ s$^{-1}$) where photosynthesis balances respiration. Numbers in bold in the last two columns indicate insufficient light to meet physiological requirements.

<table>
<thead>
<tr>
<th>Instantaneous flux (µmol m$^{-2}$ s$^{-1}$)</th>
<th>Integrated daily flux (mol m$^{-2}$ d$^{-1}$)</th>
<th>Hsat (# hours)</th>
<th>Hcomp (# hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zostera marina</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>277.03</td>
<td>11.97</td>
<td>5.49</td>
<td>11.54</td>
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<td>161.49</td>
<td>6.98</td>
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<tr>
<td>69.80</td>
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<td>2.58</td>
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<tr>
<td>33.29</td>
<td>1.44</td>
<td>-5.04</td>
<td>1.01</td>
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<tr>
<td>10.93</td>
<td>0.47</td>
<td>-6.01</td>
<td>0.04</td>
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<tr>
<td>4.21</td>
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<td>-0.25</td>
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<td>0.00</td>
<td>-6.48</td>
<td>-0.43</td>
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<tr>
<td><strong>Halodule wrightii</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>248.20</td>
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</tr>
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<td>0.00</td>
<td>-6.48</td>
<td>-0.43</td>
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Table 2: Light data from 1m and 1.5m deep sensors collected at station 6 from 2002-03, expressed as mean daily integrated irradiance (mol m\(^{-2}\) d\(^{-1}\)) each month, and the number of hours (Hsat) that irradiance exceeded a saturation intensity (150\(\mu\) m\(^{-2}\) s\(^{-1}\)). The growth period of *Z. marina* is from Dec – Jun, and the growth period for *H. wrightii* is from May – Nov. Both integrated and Hsat values are about twice as great at 1m as 1.5m depths.

<table>
<thead>
<tr>
<th>Month</th>
<th>2002-03 Mol m(^{-2}) d(^{-1})</th>
<th></th>
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<tr>
<td></td>
<td>1m</td>
<td>1.5m</td>
<td>1m</td>
<td>1.5m</td>
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<tr>
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<td>1.57</td>
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<td>4.35</td>
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<td>7.00</td>
<td>4.15</td>
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<td>4.39</td>
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<td>6.99</td>
<td>3.61</td>
<td>4.65</td>
<td>2.31</td>
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<tr>
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<td>1.86</td>
<td>0.36</td>
<td></td>
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<td>8.35</td>
<td>3.79</td>
<td>5.04</td>
<td>2.52</td>
<td></td>
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</tbody>
</table>
LIST OF FIGURES

Figure 1: A) Conceptual diagram of light attenuation down the water column (PLW), and the relative contributions of turbidity, chlorophyll $a$, and color to attenuation. Additional attenuation can occur at the leaf surface due to epiphyte fouling (PLL), which occurs primarily under heavily eutrophic conditions. B) Graphical representation of the bio-optical model. Components of light attenuation in the water column are presented along the axes. Median concentrations for one sample are plotted on this graph and compared to a minimum-light water quality requirement for a given depth (line of constant attenuation). Target minimum water-clarity requirements for seagrass survival are found at the intersection of vectors perpendicular to the axes or the origin from the median sample concentration. The target concentrations in this figure suggest that both TSS and chl $a$ need to be reduced to meet the minimum light requirements of this seagrass species.

Figure 2: Map of nine sampling locations in North River, a small estuary in the southern Pamlico Sound region of North Carolina. Seagrass beds are indicated by shaded polygons. 1 and 2m depths are indicated by the dashed contour lines.

Figure 3: Selected results from our monthly water-quality monitoring program in North River. Time-Space contour plots of temperature, salinity, chl $a$ concentration, and turbidity (NTU) measured monthly from Sept 2002 to Sept 2003. Sites are number from downstream (1) to most upstream (9). Insets are same data plotted as 3-D wireframe plots.
Figure 4: The effects of chlorophyll and turbidity on measured light attenuation with depth ($K_d$) at each of the nine stations. Filled symbols are samples collected during flood tide, open symbols are ebb-tide samples. For seagrass survival $K_d$ should not exceed 1.5, i.e., 22% of surface light reaches the bottom (dotted bar). Seagrass beds are found in North River between stations 2-6. The gray arrow indicates relative abundance of seagrasses.

Figure 5: Median monthly water quality conditions plotted for North River, based on the bio-optical model. Symbol colors indicate season (grey shadow = winter, light grey = spring and fall, black = summer), symbol shape indicates location within North River. Additionally, the North Carolina state’s estuarine water-quality criteria of 40mg m$^{-3}$ chl $a$ and 25 NTU are shown (black box). The bio-optical model was used to predict water-quality criteria for survival of seagrass to 1m depth in Chesapeake Bay (dashed line) and North River (solid line). The different slopes reflect regional differences in sediments and phytoplankton.

Figure 6: Water temperatures recorded in the indoor tank during the two light gradient experiments. Water temperatures were recorded at half hour time intervals in the highest light (tank1) and lowest light (tank6) treatments.

Figure 7: Plant growth and photosynthesis responses for $Z$. marina and $H$. wrightii in the seven irradiance treatments (units of $\mu$mol m$^{-2}$ s$^{-1}$) in the light gradient experiment. Selected variables shown are: mean number of shoots per plant (n=18), length of longest leaf measured as a proxy for canopy height, leaf area (single surface), and photosynthesis yield ratio (Fv/Fm).
Figure 8: Results of PEA derived JIP-test analyses on *H. wrightii* leaves of different ages, L1 = youngest, L3=oldest, Brn and Blk denote dead leaves in increasing stages of decomposition: A) Relationship between the relative number of active PSII reaction centers the performance index. B) Relationship between the probability that an electron will be transported beyond quinone A and the performance index.

Figure 9: Results of JIP-test on *Z. marina* plants at two months and one year age: A) Relationship between the relative number of active PSII reaction centers per unit area and the performance index. B) Relationship between the probability that an electron will be transported beyond quinone A and the performance index.
FIGURES

Fig. 1

Fig. 2
Fig. 4

Fig. 5
Fig. 6
Fig. 7
Fig. 8

Fig. 9